

Mammalian In vivo Assays for
Aneuploidy in Female Germ Cells

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16. ABSTRACT A committee of scientists was established by the U.S. Environmental Protection Agency to appraise the current state of aneuploidy test methodology, to compile and analyze published data on the chemical induction of aneuploidy, and to provide guidance for additional test development and validation. The committee was organized into 9 working groups, representing different eukaryotic organisms ranging from fungi to whole mammals. The Environmental Mutagen, Carcinogen, and Teratogen Information Program at Oak Ridge National Laboratory identified and provided literature to the working groups. The 9 reports were prepared by the individual working groups. The reports include: (1) descriptions of the available testing approaches and general principles to be followed for protocol development, (2) criteria for evaluating data, and (3) recommendations for future test development and basic research. The evaluated results are presented in tables. For the positive and negative calls, the lowest effective dose tested or the highest ineffective dose tested, respectively, and the range of concentrations tested are provided. An overall summary report was prepared that summarizes the conclusions and recommendations of the 9 working groups and the chemical data base. It is hoped that these reports will provide a framework for further test development and validation of aneuploidy assays.		
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Mammalian in vivo assays for aneuploidy in female germ cells *

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Summary

This paper presents an evaluation of and offers recommendations for assays to detect chemically induced aneuploidy in mammalian female germ cells. 72 papers on female germ cell aneuploidy, published from 1970 to 1984, were reviewed. 28 papers were selected for critical evaluation; the other 44 papers were rejected according to pre-established criteria. Salient points emerging from the information reviewed allow an assessment of the current status of mammalian female germ cell tests for aneuploidy.

* Report of the Aneuploidy Data Review Committee (Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC).

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- (a) The majority of data have been obtained by analyzing metaphase II mouse oocyte chromosomes following superovulation.
- (b) Various classes of chemicals were administered usually around the time of ovulation.
- (c) Dose-response relationships have not been obtained for the majority of chemicals evaluated.
- (d) The method of data reporting and analysis usually was not conducive to comparisons among different studies.
- (e) Few of the 16 chemicals studied can be regarded as negative for their ability to induce aneuploidy, whereas an even smaller number should be considered as positive.

Certainly, a need exists to identify the chemicals and the dosages that could increase the incidence of aneuploidy in mammalian female germ cells. Obtaining such data definitely is feasible in cytogenetic laboratories. However, the mammalian female germ cell aneuploid assay should not be perceived as a rapid, inexpensive, routine procedure. The assay is capable of detecting aneuploidy following anaphase I when metaphase II oocytes are studied and following anaphases I and II when first-cleavage zygotes are studied.

(1) Introduction

Aneuploidy in humans arises primarily in maternal germ cells. The extra chromosome is of maternal origin in about 80% of liveborn cases of trisomy 21 (Juberg and Mowrey, 1983; Chandley, 1981) and in an even greater proportion of trisomic spontaneous abortions (Hassold et al., 1984; Meulenbroek and Geraedts, 1982). An experimental system to study induction of aneuploidy in female germ cells of mammals has, therefore, great relevance to the human situation.

Increasing maternal age is associated with elevated frequencies of certain human trisomies and various hypotheses have been advanced to account for this relationship (Sankaranarayanan, 1979; Chandley, 1982). Agents such as X-rays (Uchida, 1979) and chemicals (Mikkelsen et al., 1980), as well as genetic predisposition for nondisjunction (Alfi et al., 1980), have been postulated to enhance nondisjunction in man. Although much has been written about aneuploidy, very little is known for certain about its causes.

Previous reports have assessed the ability of certain chemicals to induce heritable translocations in mice (Generoso et al., 1980) or chromosome aberrations in mammalian cells *in vivo* or *in vitro* (Preston et al., 1981). This report will focus on whole chromosome aneuploidy (numerical aberrations) rather than structural aberrations in mammalian female germ cells. The importance of utilizing female germ cells in mutagenicity stud-

ies has been reviewed in several publications (Röhrborn et al., 1977; Adler and Brewen, 1982; Brewen and Preston, 1982).

A total of 72 published papers involving aneuploidy in mammalian female germ cells were reviewed. Twenty-eight were accepted for critical review while the other 44 were rejected. A two-step process was employed for identifying acceptable papers. The criteria associated with the first step are presented in the general introduction (Dellarco et al., this volume). Criteria specific for the mammalian female germ cell aneuploid assay (second step) include:

(1) True aneuploidy data, not endoreduplication, satellite association, polyploidy, chromosomal structural aberrations, chromosome separation including univalents, C-mitosis, atretic oocytes, or, other cytologic events not specifically hyperploidy or hypoploidy.

(2) Either concurrent or historical control data.

Those papers not eliminated during the first or second steps were critically evaluated.

(2) Test description

Aneuploidy may be defined as a condition in which a cell or an organism possesses fewer or more chromosomes than an exact multiple of the haploid number. Although aneuploidy is the quantitative and point measured in this assay, the mechanisms of its production are not addressed.

The mammalian female germ cell aneuploid

assay involves the administration of a chemical to female rodents (mice or hamsters) prior to ovulation and subsequent cytogenetic analysis of metaphase II oocytes or early embryonic cells. Rodents between 8 and 12 weeks of age are superovulated with pregnant mare's serum (PMS) and human chorionic gonadotrophin (HCG), and the chemical treatment is given intraperitoneally about the time of HCG injection. Approximately 16 h post HCG, metaphase II (MII) oocytes are collected from the oviduct and are processed for cytogenetic analysis according to the basic method of Tarkowski (1966). For analysis of embryonic cells, females are mated following superovulation and chemical treatment. Embryos are collected either at first cleavage or at different times up to 16 days of gestation and are processed for cytogenetic analyses.

Different cytogenetic errors are expected, depending on the cell type analyzed. Table 1 lists conditions observable at various stages of female germ cell development. Cytogenetic defects induced in early stages may be transmitted to later cell types (e.g., nondisjunction at meiotic metaphase I can be detected by analysis of first-cleavage zygotes or later embryonic stages). For chronic treatments in which several cell types are exposed to a chemical, cytogenetic errors associated with each of the respective cell types may be expected. Information about the cell types exposed relative to the cell types analyzed is required to interpret the assay. If primary oocytes are exposed to a chemical that is rapidly eliminated and the chromosomes analyzed in F_1 progeny, the assay is expected to be less sensitive than if chromosomes from secondary oocytes were studied because

TABLE 1
CYTOGENETIC BASIS OF TESTS FOR DETECTING ANEUPLOIDY IN FEMALE GERM CELLS

Cell/organism analyzed	Primary end points	Possible cytogenetic errors
Oogonia	Numerical aberrations at mitotic metaphase	Chromosome gain/loss during mitosis; defective centromere division
Primary oocyte	Univalent and bivalent counts at metaphase I; chiasmata counts during prophase	Nonconjunction of homologues; reduced crossing over
Secondary oocyte	Numerical aberrations at metaphase II; single unpaired chromatids	Nondisjunction of homologues or premature centromere division during meiosis I
First-cleavage zygote	Numerical aberrations in female pronucleus; single unpaired chromatids	Nondisjunction of dyads or premature centromere division during meiosis II
Embryonic cells	Numerical aberrations at metaphase	Chromosome gain/loss during mitosis; segregation errors during parental meiosis
F_1 progeny	Numerical aberrations at metaphase; phenotypic expression of X-linked genetic markers	Chromosome gain/loss during mitosis; segregation errors during parental meiosis; chromosome deletions

aneuploidy can cause embryonic death, and the cells analyzed are removed in time from those initially exposed.

A suggested testing protocol for MII oocytes and first-cleavage zygotes is presented. These two cell types are preferred for analysis of meiotic errors over later embryonic stages (8–16-day-old embryos) because aneuploidy induced in primary or secondary oocytes can be directly quantified without the possibility of cell loss or mosaic aneuploidy induced during subsequent mitotic divisions.

(2.1) Species

Mice, Syrian hamsters, and Chinese hamsters have been used.

(2.2) Strain and age

Females of various strains and ages have been used, and specific recommendations would be inappropriate because comparative data are lacking. However, the use of young females is recommended in order to avoid the possibility of advanced maternal age influencing the incidence of aneuploidy. Treated groups and controls should be concordant regarding strain and age.

(2.3) Superovulation

To increase the number of oocytes obtained and induce ovulation at prescribed times, female mice may be injected i.p. with 2–5 IU PMS and, 48 h later, with 2–5 IU HCG. Hansmann and Probeck (1979) reported that these hormones did not increase the incidence of aneuploidy in MII oocytes in mice, Chinese hamsters, or Syrian hamsters. Significant increases of hyperploid oocytes were not detected after hormonally stimulated ovulation in 3 different strains of mice (Hansmann, 1978; Hansmann and El-Nahass, 1979). Superovulation also was shown not to increase the incidence of aneuploidy among young versus old mice of 2 different strains (Golbus, 1981). Although the majority of investigators used low (2–5 IU) doses of gonadotrophins to induce ovulation in rodents, increased hyperploidy has been reported in NMRI/HAN $\times$ C57/bl F₁ hybrid mice, but not in 2 other hybrids or in 3 parental strains following 10 IU of PMS and HCG (Hansmann and Jenderny, 1983). The possibility of certain

species being especially sensitive to gonadotrophin-induced aneuploidy may be exemplified by the ovulation of aneuploid '*Phodopus sungorus*' oocytes following increased doses of gonadotrophins (Hansmann et al., 1980).

(2.4) Negative controls

A random sample of animals of similar age and genetic background as the treated animals should serve as concurrent controls. These controls should be superovulated in the same manner as treated females and injected with the same amount of carrier solvent that treated animals receive. Isotonic saline or distilled water have been used most commonly.

Recently, Bond and Chandley (1983) compiled the results of various independent studies concerning the incidence of spontaneous aneuploidy in mouse metaphase II oocytes. When 5088 oocytes from young (2–6 months) mice, either superovulated (4324 oocytes) or spontaneously ovulated (764 oocytes), were studied, the incidence of aneuploidy (twice the hyperploid frequency) ranged from 0.0 to 2.3% with a mean of 0.7%. On the other hand, the incidence of aneuploidy in 1147 metaphase II oocytes from older mice (7–18.5 months) ranged from 1.2 to 6.2% with a mean of 2.8%. When 4373 oocytes from young or old mice were cultured in vitro, the incidence of aneuploidy ranged from 0.0 to 12.3% with a mean of 2.5%. Spontaneous aneuploidy appears least variable when oocytes from young mice are obtained directly from the oviducts without in vitro culture.

Information on the frequency of spontaneous aneuploidy in species other than mice is more limited. Hansmann and Probeck (1979) reported that one hyperploid metaphase II was detected among 455 Syrian hamster oocytes; no hyperploids were found among 455 Chinese hamster metaphase II oocytes.

(2.5) Positive controls

Incorporation of a positive control into the experimental protocol for detecting aneuploidy in female germ cells is recommended. On the basis of data presently available, specific recommendations for a positive control agent seem premature. Certain clastogens could be used to demonstrate that germ cells incurred cytogenetic damage, but the

relationship between clastogens and chemicals capable of inducing aneuploidy has not been established.

(2.6) Chemical treatment

Additional data are needed to determine the most appropriate time to administer test chemicals. A single, intraperitoneal injection 10–14 h prior to the time of expected ovulation is currently recommended. If superovulation is employed, it is convenient to administer the chemical treatment at the time of HCG injection. The test chemical solution should be freshly prepared in a defined solvent prior to injection. Although dose–response effects are not expected necessarily for aneuploidy data, administration of at least 3 different dosages of the test compound increases the probability of identifying a sensitive concentration range (if present) and determining whether a dose response exists. The highest dose should be one that induces significant toxicity and the lowest at least an order of magnitude less than the highest dose. It would be most appropriate to base the doses on toxicity to the oocytes themselves, but this is both time consuming and expensive. The value of the additional information from such an approach should be taken into account.

(2.7) Cell harvest

Since post-ovulatory oocytes remain in metaphase II until fertilization, a metaphase-blocking agent is not required for MII oocyte analysis. Approximately 4–6 h post ovulation or 16 h after HCG, mouse oocytes can be collected by tearing or piercing the ampullar portion of the oviduct and allowing the cumulus mass containing the oocytes to exude into a basal salt solution or liquid medium. The cumulus mass is then placed into a hyaluronidase solution to liberate the oocytes. Subsequent preparative steps include exposure to a hypotonic solution (e.g., 1% sodium citrate for 20 min) and fixation in 3:1 methanol–glacial acetic acid. Air-dried slides are prepared and chromosomes are C-banded (Summer, 1972) to locate the centromeres. Besides the basic technique of Tarkowski (1966), the mass isolation and processing method of Payne and Jones (1975) should be considered. Cytogenetic methods may appear to be without complications,

but time and effort are required to develop the technical proficiency that consistently yields a sufficient number of cells suitable for analysis.

For cytogenetic analysis of first-cleavage zygotes, two additional steps are required. The females are mated and a metaphase-blocking agent is used to arrest the pronuclei at metaphase. One feasible protocol for mice involves administering HCG and test or control compounds to females at 14.00 h (PMS given 48 h earlier) and arranging single-pair matings. The following morning (09.00 h), females are checked for copulatory plugs and 2×10^{-3} M colchicine is given to each plugged female at 16.00 h. First-cleavage zygotes are flushed from the oviduct at about 09.00 h on the subsequent day. Cell processing and slide preparation are essentially the same as for MII oocytes. Superovulation and cell harvest protocols differ for species other than mice and reference to original papers is recommended (Kamiguchi et al., 1976; Basler, 1978; Watanabe et al., 1979; Mizoguchi and Dukelow, 1980, 1981).

(2.8) Chromosome analysis

Cells containing chromosomes that are unduly scattered, clumped, overlapped, or otherwise ambiguous should not be analyzed. C-banding of both MII oocytes and first-cleavage zygotes is strongly encouraged to objectively quantify the centromeric number. Coded slides are required so that the microscopist is unaware of which treatment group is being analyzed. At least 100 cells should be analyzed per experimental group, including concurrent controls; 20 animals per experimental group is the recommended number to enhance the probability of obtaining a representative sample. The number of centromeres within each cell should be counted and recorded; only cells that allow an unequivocal count of centromeres should be used.

(3) Interpretation of data

Incidences of hypoploid and hyperploid cells should be reported separately. We recommend that hypoploidy be scored as cells missing only one centromeric chromosome and hyperploidy as cells with one additional chromosome; additional categories can be recorded if needed. Ambiguity in

TABLE 2
RESULTS FOR CHEMICALS EVALUATED IN MAMMALIAN FEMALE GERM CELLS IN VIVO

Chemical (CAS No.)	Cell type analyzed (species) ^a	Conclusions ^b			LEDT ^c (mg/kg)	HIDT ^d (mg/kg)	Dosage (mg/kg)	Criteria for I calls ^e	References
		Hyper- ploidy	Hypo- ploidy	Struc- aberra- tions					
Acriflavine (8048-52-0)	MII oocyte (M)	I	I	I			0, 2	C, D	Basler et al. (1977)
Benzof <i>a</i> pyrene (50-32-8)	F ₁ progeny (M)	0	I	0			0, 100, 200, 300	B	Russell and Russell (1978)
Cadmium chloride (10108-64-2)	MII oocyte (M)	I	I	-			0, 3, 6	C	Shimada et al. (1976)
Cadmium chloride	MII oocyte (M)	+	I	-	6		0, 3, 6	D	Watanabe et al. (1977)
Cadmium chloride	MII oocyte (SH)	+	I	0	4		0, 1, 2, 4	D	Watanabe et al. (1979)
Cadmium chloride	blastocyst (M)	+	+	0	1.5		0, 1.5, 3		Watanabe and Endo (1982)
4-Chloromethylbiphenyl (1667-11-4)	MII oocyte (M)	-	-	0		100	0, 50, 100		Brook (1982)
Colchicine (64-86-8)	MII oocyte (CH)	+	+	0	3		0, 3		Sugawara and Mikamo (1980a)
Colchicine	MII oocyte (CH)	I	I	0			0, 3	C	Sugawara and Mikamo (1980b)
Colchicine	MII oocyte (CH)	I	I	0			0, 3	A	Mikamo and Sugawara (1980)
Cyclophosphamide (50-18-0)	MII oocyte (M)	+	+	+	145		0, 145		Röhrborn and Hansmann (1971)
Cyclophosphamide	MII oocyte (M)	I	I	+			0, 30, 50, 145	C	Hansmann (1974)
Cyclophosphamide	MII oocyte (M)	+	I	-	150		0, 150	D	Becker and Schöneich (1982)
Ethanol (64-17-5)	1-cell embryo (M)	I	I	0			0%, 10%, 12.5%, 15%	A	Kaufman (1983)
Haloethane (151-67-7)	16-day embryo (M)	-	-	0		1%	0%, 1%		Basler and Röhrborn (1981)
Isoniazid (54-85-3)	MII oocyte (M)	-	-	-		25	0, 25		Röhrborn et al. (1978)
Mercuric chloride (7487-94-7)	MII oocyte (SH)	-	-	-		12.8	0, 6.4, 12.8		Watanabe et al. (1982)
Methotrexate (59-05-2)	MII oocyte (M)	+	+	+	200		0, 200		Röhrborn and Hansmann (1971)

Methotrexate	Pronuclei (M)	I	I	I	I	0, 200	A	Hansmann (1973)
Methotrexate	2-cell embryo (M)	I	I	I	I	0, 200	A	Hansmann (1973)
Methotrexate	Morulae (M)	I	I	I	I	0, 200	A	Hansmann (1973)
Methotrexate	MII oocyte (M)	+	+	+	+	0, 15, 30, 50, 100, 200		Hansmann (1974)
Methotrexate	MII oocyte (M)	-	+	+	0	0, 200		Gates et al. (1981)
Methylmercuric chloride (115-09-3)	MII oocyte (SH)	I	I	-	-	0, 6.4, 12.8	D	Watanabe et al. (1982)
Methylmercuric chloride	MII oocyte (SH)	+	+	+	-	0, 10		Mailhes (1983)
Norethindrone acetate (51-98-9)	MII oocyte (M)	+	+	+	0	0, 1, 10		Röhrborn and Hansmann (1974)
Pyrimethamine (58-14-0)	5-day embryo (R)	+	I	-	5	0, 2, 5	D	Baranov and Chebotar (1980)
Pyrimethamine	MII oocyte (R)	I	I	I	I	0, 2, 5, 25	D	Chebotar (1980)
Triaziquone (68-76-8)	2-cell embryo (M)	I	I	I	I	0, 0.25	A	Röhrborn et al. (1971)
Triaziquone	MII oocyte (M)	I	I	I	I	0, 0.25, 1.0	A	Röhrborn and Hansmann (1971)
Triaziquone	Morulae (M)	+	+	+	+	0, 0.25		Hansmann and Röhrborn (1973)
Triaziquone	MII oocyte (CH)	I	I	+	+	0, 0.25, 1.0	A	Hansmann et al. (1974)
Triaziquone	9.5 and 13.5-day embryo (M)	0	-	-	-	0, 0.25		Basler et al. (1976)
Triaziquone	MII oocyte (SH)	-	0	+	+	0, 0.03, 0.25, 2.0		Basler (1978)
Triaziquone	MII oocyte (M)	+	+	+	-	0, 1.0		Becker and Schöneich (1982)
Triaziquone	1-cell embryo (M)	+	+	+	+	0, 0.25, 1.0		Becker and Schöneich (1982)
2,4,5-Trichlorophenoxyacetic acid (93-76-5)	MII oocyte (R)	I	I	I	I	0, 200, 400	D	Chebotar (1980)

^a M, mouse (*Mus musculus*); R, rat (*Rattus norvegicus*); CH, Chinese hamster (*Cricetulus griseus*); SH, Syrian hamster (*Mesocricetus auratus*).

^b +, positive; Based on author's statistical analysis ($P \leq 0.05$) or reviewer's evaluation of data. -, negative; Based on author's statistical analysis ($P > 0.05$) or reviewer's evaluation of data. I, inconclusive; Based on author's and/or reviewer's judgement of small sample size, use of inappropriate data (e.g., total aneuploid cells instead of hyperploid cells), or possible uncontrolled biologic variation (e.g., superovulation of treated but not control animals). 0, no information available from the paper.

^c LEDT, lowest effective dose tested, for positive test results. A single dose was considered equivalent to the LEDT if the observed response was at least twice the control level.

^d HIDT, highest ineffective dose tested, for negative test results. If there was evidence of extreme toxicity, the next-to-highest dose was used. A single dose tested with a negative result was considered equivalent to the HIDT.

^e A, insufficient sample size; B, only hypoploid data presented (X chromosome loss); C, conclusions based on 'total aneuploidy' data only; D, uncertain statistical significance.

data interpretation can result if all cells with different numbers of missing chromosomes are reported under the general category of hypoploidy. A proportion of 'grossly' hypoploid cells no doubt result from the technical artifact of excess chromosome scatter during slide preparation. Because aneuploidy can result from failure of a chromosome to be incorporated into a daughter cell, the incidence of hypoploidy should be recorded. This event would not generate a concomitant hyperploid cell, and the incidences of hypoploidy and hyperploidy would differ.

(3.1) Presentation of data

The following data elements are recommended for controls and for each treatment group:

- (a) number of females used;
- (b) number of oocytes or zygotes collected;
- (c) number of cells analyzed;
- (d) number of euploid cells;
- (e) number of hyperploid cells (identify ploidy);
- (f) number of hypoploid cells (identify ploidy);
- (g) number of each specific type of chromosomal structural aberrations (if detected).

The most appropriate method for statistical analysis of data should be determined prior to data collection, during the experimental design part of the project. Statistical tests that have been utilized for aneuploidy data include chi-square, Fisher's Exact test, and Analysis of variance. The most relevant statistical analysis will depend on the specific experimental design. Most important for aneuploidy studies is whether the proportion of hyperploid cells (number of hyperploid cells/number of cells analyzed) in the treated group(s) differs ($P < 0.05$) from that proportion in the controls. It is appropriate to double the incidence of hyperploid cells and statistically compare this figure which the same parameter in controls; doubling of hyperploid cells assumes the concomitant loss of chromosomes to give hypoploid cells.

When presenting structural chromosomal aberration data, specific aberrations should be expressed as the frequency per cell. Structural aberrations should be categorized as chromosome- or chromatid-type and further subdivided into deletions and exchanges within each category, because different aberrations have different dose relations and genetic consequences. Dose-response rela-

tionships can be evaluated by appropriate regression analyses, which may yield insight regarding induction kinetics, etiologic mechanisms, and threshold effects. For consistency in data reporting and interpretation, analysis of structural chromosomal aberrations should follow published criteria (Buckton and Evans, 1973; Savage, 1976).

(4) Test performance

The efficacy of the whole mammal female germ cell aneuploid assay was evaluated using data from 28 accepted papers that contained information on 16 different chemicals (Table 2). Chemicals considered positive for hyperploidy induction were cadmium chloride, colchicine, cyclophosphamide, methotrexate, methylmercuric chloride, norethindrone acetate, pyrimethamine, and triaziquone. Conversely, 4-chloromethylbiphenyl, halothane, isoniazid, mercuric chloride, and triaziquone were considered negative. Results for acriflavine, benzo[a]pyrene, ethanol, and 2,4,5-trichlorophenoxyacetic acid were judged inconclusive (I) or insufficient information was available from the papers. The I calls were based on (a) insufficient sample size, (b) only hypoploid data or total aneuploidy data only, or (c) uncertain statistical significance. The fact that several chemicals were given more than one call can be attributed to differences among experimental protocols reported in different publications.

The mammalian female germ cell aneuploidy assay appears sufficiently sensitive and certainly is relevant for assessing aneuploidy risks in humans if sound experimental protocols are followed.

(5) Conclusions

Cytogenetic analysis of MII oocytes allows for a direct quantitative determination of aneuploidy following anaphase I. It provides no information about anaphase II events. This particular assay presently is recommended because the majority of aneuploidy events in humans occur during female anaphase I (Jacobs and Hassold, 1980; Mikkelsen et al., 1980) and because breeding of the experimental animals is not required. Analysis of first-cleavage zygote chromosomes permits determination of aneuploidy following anaphase II.

Since most chemical mutagens require an intervening round of DNA synthesis for structural chromosomal damage to be manifested, analysis of first-cleavage zygotes is recommended when clastogenic events are also of interest. A more comprehensive protocol, including cytogenetic analysis of both MII oocytes and first-cleavage zygotes, would provide direct evidence of aneuploidy occurring in both meiosis I and II, as well as detection of structural aberrations in the first metaphase following DNA synthesis. The mammalian female germ cell aneuploid assay appears relevant for obtaining data extrapolation to humans. Practical drawbacks are the expense of animal procurement and maintenance and the high degree of technical skill required for preparing quality slides.

Before validation of the mammalian female germ cell assay, agreement on basic protocols and identification of positive control chemicals that consistently increase the incidence of aneuploidy are needed. Validation requires testing of a broad range of chemicals in different laboratories. Principal factors in protocol standardization include: scoring criteria, method of data presentation and statistical analysis, and time to administer treatment. Once validated, this assay should be used as a second-tier test for genetic risk assessment of chemicals initially detected in a primary screening system. There is additional need for research addressing the mechanism(s) of aneuploidy induction. Identification of chemicals capable of significantly increasing the incidence of aneuploidy in mammalian germ cells (in vivo) and understanding their mode of action are of great importance in identifying potential risks to human populations.

References

- Adler, I., and J.G. Brewen (1982) Effects of chemicals on chromosome aberration production in male and female germ cells, in: F.J. de Serres and A. Hollaender (Eds.), *Chemical Mutagens: Principles and Methods for their Detection*, Vol. 7, Plenum, London.
- Alfi, O.S., R. Chang and S.P. Azen (1980) Evidence for genetic control of nondisjunction in man, *Am. J. Hum. Genet.*, 32, 477-483.
- Baranov, V.S., and N.A. Chebotar (1980) Mutagenic effects of preovulatory administration of pyrimethamine on preimplantation rat embryos, *Tsitol. Genet.*, 14, 20-24.
- Basler, A. (1978) Timing of meiotic stages in oocytes of the Syrian hamster (*Mesocricetus auratus*) and analysis of induced chromosome aberrations, *Hum. Genet.*, 42, 67-77.
- Basler, A., and G. Röhrborn (1981) Lack of mutagenic effects of halothane in mammals in vivo, *Amesthesiology*, 55, 143-147.
- Basler, A., B. Buselmaier and G. Röhrborn (1976) Elimination of spontaneous and chemically induced chromosome aberrations in mice during early embryogenesis, *Hum. Genet.*, 33, 121-130.
- Basler, A., M. Brucklacher, F. Nobis and G. Röhrborn (1977) Comparative investigations with trypanflavin in metaphase-II oocytes and in dominant lethal assays, *Hum. Genet.*, 40, 87-92.
- Becker, K., and J. Schöneich (1982) Expression of genetic damage induced by alkylating agents in germ cells of female mice, *Mutation Res.*, 92, 447-464.
- Bond, D.J., and A.C. Chandley (1983) *Aneuploidy*, Oxford Monographs on Medical Genetics No. 11, Oxford University Press, London.
- Brewen, J.G., and R.J. Preston (1982) Cytogenetic analysis of mammalian oocytes in mutagenicity studies, in: T.C. Hsu (Ed.), *Cytogenetic Assays of Environmental Mutagens*, Allanheld, Osmun.
- Brook, J.D. (1982) The effect of 4CMB on germ cells of the mouse, *Mutation Res.*, 100, 305-308.
- Buckton, K.E., and H.J. Evans (1973) *Methods for the Analysis of Human Chromosome Aberrations*, World Health Organization, Geneva.
- Chandley, A.C. (1981) The origin of chromosomal aberrations in man and their potential for survival and reproduction in the adult human population, *Ann. Génét.*, 24, 5-11.
- Chandley, A.C. (1982) The origin of aneuploidy, in: B. Bonne-Tamir (Ed.), *Progr. Clin. Biol. Res.*, 103B, Human Genetics, Part B, Medical Aspects, Proc. Sixth Int. Congr. Human Genetics, Liss, New York.
- Chebotar, N.A. (1980) Cytogenetic and morphogenetic changes in oogenesis and embryogenesis of albino rats induced by chlordin and 2,4,5-trichlorophenoxyacetic acid in the preovulation phase of meiosis, *S.O.G.E.B.*, 16, 781-787.
- Gates, A.H., C.H. Donaldson and M.D. Levy (1981) Oocyte aneuploidy screening using superovulating prepubertal mice: Effect of methotrexate, *Teratology*, 24, 321-327.
- Generoso, W.M., J.B. Bishop, D.G. Gosslee, G.W. Newell, C.J. Sheu and E. von Halle (1980) Heritable translocation test in mice, *Mutation Res.*, 76, 191-215.
- Golbus, M.S. (1981) The influence of strain, maternal age, and method of maturation on mouse oocyte aneuploidy, *Cytogenet. Cell Genet.*, 31, 84-90.
- Hansmann, I. (1973) Induced chromosomal aberrations in pronuclei, 2-cell stages and morulae of mice, *Mutation Res.*, 20, 353-367.
- Hansmann, I. (1974) Chromosome aberrations in metaphase II oocytes, Stage sensitivity in the mouse oogenesis to amethopterin and cyclophosphamide, *Mutation Res.*, 22, 175-191.
- Hansmann, I. (1978) The induction of non-disjunction in mammalian oogenesis, in: H.J. Evans and D.C. Lloyd (Eds.), *Mutagen-Induced Chromosome Damage in Man*, Edinburgh University Press, Edinburgh.

- Hansmann, I., and E. El-Nahass (1979) Incidence of nondisjunction in mouse oocytes, *Cytogenet. Cell Genet.*, 24, 115-121.
- Hansmann, I., and J. Jenderny (1983) The genetic basis of non-disjunction: Increased incidence of hyperploidy in oocytes from F₁ hybrid mice, *Hum. Genet.*, 65, 56-60.
- Hansmann, I., and H.D. Probeck (1979) Detection of nondisjunction in mammals, *Environ. Health Perspect.*, 31, 161-165.
- Hansmann, I., and G. Röhrborn (1973) Chromosome aberrations in preimplantation stages of mice after treatment with triazoquinone, *Humangenetik*, 18, 101-109.
- Hansmann, I., J. Neher and G. Röhrborn (1974) Chromosome aberrations in metaphase II oocytes of Chinese hamster (*Cricetus griseus*), I. The sensitivity of the pre-ovulatory phase to triaziquone, *Mutation Res.*, 25, 347-359.
- Hansmann, I., J. Jenderny and H.D. Probeck (1980) Mechanisms of non-disjunction: Gonadotrophin-induced aneuploidy in oocytes from *Phodopus sungorus*, *Eur. J. Cell Biol.*, 22, 24.
- Hassold, T., D. Chiu and J.A. Yamane (1984) Parental origin of autosomal trisomies, *Ann. Hum. Genet.*, 48, 129-144.
- Jacobs, P.A., and T.J. Hassold (1980) The origin of chromosome abnormalities in spontaneous abortions, in: E.B. Hook and I.H. Porter (Eds.), *Human Embryonic and Fetal Death*, Academic Press, New York.
- Juberg, R.C., and P.N. Mowrey (1983) Origin of nondisjunction in trisomy 21 syndrome: All studies compiled, parental age analysis, and international comparisons, *Am. J. Med. Genet.*, 16, 111-116.
- Kamiguchi, Y., K. Funaki and K. Mikamo (1976) A new technique for chromosome study of murine oocytes, *Proc. Jpn. Acad.*, 52, 316-319.
- Kaufman, M.H. (1983) Ethanol-induced chromosomal abnormalities at conception, *Nature (London)*, 302, 258-260.
- Mailhes, J.B. (1983) Methylmercury effects on Syrian hamster metaphase II oocyte chromosomes, *Environ. Mutagen.*, 5, 679-686.
- Meulenbroek, G.H.M., and J.P.N. Geraedts (1982) Parental origin of chromosome abnormalities in spontaneous abortions, *Hum. Genet.*, 62, 129-133.
- Mikamo, K., and S. Sugawara (1980) Colchicine-induced abnormal meiotic chromosomal segregation in primary oocytes of the Chinese hamster, Part II. Anaphase lagging, *Jpn. J. Hum. Genet.*, 25, 241-248.
- Mikkelsen, M., H. Poulsen, J. Grinsted and A. Lange (1980) Nondisjunction in trisomy 21, Study of chromosomal heteromorphisms in 110 families, *Ann. Hum. Genet.*, 44, 17-28.
- Mizoguchi, H., and W.R. Dukelow (1980) Effect of timing of hCG injection on fertilization in superovulated hamsters, *Biol. Reprod.*, 23, 237-241.
- Mizoguchi, H., and W.R. Dukelow (1981) Fertilizability of ova from young or old hamsters after spontaneous or induced ovulation, *Fertil. Steril.*, 35, 79-83.
- Payne, H.S., and K.P. Jones (1975) Technique for mass isolation and culture of mouse ova for cytogenetic analysis of the first cleavage mitosis, *Mutation Res.*, 33, 247-249.
- Preston, R.J., W. Au, M.A. Bender, J.G. Brewen, A.V. Carrano, J.A. Heddle, A.F. McFee, S. Wolff and J.S. Wassom (1981) Mammalian in vivo and in vitro cytogenetic assays: A report of the U.S. EPA's Gene-Tox Program, *Mutation Res.*, 87, 143-188.
- Röhrborn, G., and I. Hansmann (1971) Induced chromosome aberrations in unfertilized oocytes of mice, *Humangenetik*, 13, 184-198.
- Röhrborn, G., and I. Hansmann (1974) Oral contraceptives and chromosome segregation in oocytes of mice, *Mutation Res.*, 26, 535-544.
- Röhrborn, G., O. Kühn, I. Hansmann and K. Thon (1971) Induced chromosome aberrations in early embryogenesis of mice, *Humangenetik*, 11, 316-322.
- Röhrborn, G., I. Hansmann and U. Buckel (1977) Cytogenetic analysis of pre- and postovulatory oocytes and pre-implantation embryos in mutagenesis of mammals, in: B.J. Kilbey, M. Legator, W. Nichols and C. Ramel (Eds.), *Handbook of Mutagenicity Test Procedures*, Elsevier/North-Holland Biomedical Press, Amsterdam.
- Röhrborn, G., V. Sezer, I.-D. Adler, A. Schmalz, I.H. Pawlowitzki and Ch. Nautsch (1978) Effects of isoniazid (INH) on the oogenesis of mice, *Hum. Genet.*, 42, 55-58.
- Russell, W.L., and L.B. Russell (1978) Use of the mouse specific-locus method to quantify the gene mutation hazard from mutagens associated with non-nuclear energy technologies, EPA three-year summary.
- Sankaranarayanan, K. (1979) The role of non-disjunction in aneuploidy in man: An overview, *Mutation Res.*, 61, 1-28.
- Savage, J.R.K. (1976) Classification and relationships of induced chromosomal structural changes, *J. Med. Genet.*, 13, 103-122.
- Shimada, T., T. Watanabe and A. Endo (1976) Potential mutagenicity of cadmium in mammalian oocytes, *Mutation Res.*, 40, 389-396.
- Sugawara, S., and K. Mikamo (1980a) An experimental approach to the analysis of mechanisms of meiotic non-disjunction and anaphase lagging in primary oocytes, *Cytogenet. Cell Genet.*, 28, 251-264.
- Sugawara, S., and K. Mikamo (1980b) Colchicine-induced abnormal meiotic chromosomal segregation in primary oocytes of the Chinese hamster, Part I. Nondisjunction, *Jpn. J. Hum. Genet.*, 25, 235-240.
- Summer, A.T. (1972) A simple technique for demonstrating centromeric heterochromatin, *Exp. Cell Res.*, 75, 304-306.
- Tarkowski, A.K. (1966) An air-drying method for chromosome preparations from mouse eggs, *Cytogenetics*, 5, 394-400.
- Uchida, I.A. (1979) radiation-induced nondisjunction, *Environ. Health Perspect.*, 31, 13-18.
- Watanabe, T., and A. Endo (1982) Chromosome analysis of preimplantation embryos after cadmium treatment of oocytes at meiosis, I. *Environ. Mutagen.*, 4, 563-567.
- Watanabe, T., T. Shimada and A. Endo (1977) Mutagenic effects of cadmium on the oocyte chromosomes of mice, *Jpn. J. Hygiene*, 32, 472-481.
- Watanabe, T., T. Shimada and A. Endo (1979) Mutagenic effects of cadmium on mammalian oocyte chromosomes, *Mutation res.*, 67, 349-356.
- Watanabe, T., T. Shimada and A. Endo (1982) Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female golden hamsters, *Teratology*, 25, 381-384.